A Novel Approach to Prove Bacterial Leakage of Implant-Abutment Connections In Vitro

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Bacterial leakage from the implant-abutment-interface (IAI) is suspected of contributing to the development of peri-implantitis. The aim of the study was to develop a straightforward test setup to evaluate the bacterial leakage of the IAI of 2-piece implant systems under laboratory conditions. A test suspension of *Enterococcus faecium* was injected into 7 implants (PerioType Rapid Implants) prior to abutment fixation. The IAI was covered by kanamycin aesculn azide agar (KAAA), which serves as an optical indicator for *E. faecium*. The specimens were cyclically loaded with a force of 120 N for up to 1 000 000 cycles in a universal testing machine in accordance with the ISO 14801:2007 standard. The color change of the KAAA was recorded. Three of the 7 implants showed bacterial leakage before the cyclic loading test started. The bacterial tightness of the IAIs of the 4 remaining implants lasted for 35 680 ± 22 467 cycles on average. The experimental setup at hand provides the means for a straightforward evaluation of the bacterial tightness of the IAI of 2-piece dental implants.

Key Words: bacterial leakage, Enterococcus faecium, dental implants, dental implant-abutment interface, peri-implantitis, chewing simulation

INTRODUCTION

Dental implants serve as artificial dental roots for supporting single crowns, fixed dental prostheses (FDPs), and removable dental prostheses (RDPs). Most often, 2-piece implant systems are employed. The first part, the implant body, is anchored in the bone (osseointegrated). After the healing phase, a second component, the meso-structure (abutment), is connected to the implant body by means of a central fixation screw. The abutment itself serves as a firm anchor for the actual restoration.[1] One-piece implant systems differ from 2-piece dental implants in that implant and abutment are a single part. In contrast to 1-piece implant systems, 2-piece systems allow for a submerged healing procedure of the implant beneath the mucosa. This protocol is beneficial when implant placement and bone augmentation are performed simultaneously because it mitigates the risk of bacterial contamination of the augmentation material.[2] Furthermore, the 2-stage procedure helps to minimize the risk of the implant being exposed to stress too early, which might impede a successful osseointegration. Moreover, due to their prosthetic versatility, 2-piece systems cover a maximum range of indications.

After finalization of the prosthetic treatment, a design-dependent persistent micro-gap ranging from 0 to 60 μm at the implant-abutment-interface (IAI) cannot always be avoided.[3–5] This cavity leads to a bacterial colonization of the interior of the implant.[3,6] Additionally, the existence of such a gap enables micromovements between abutment and implant.[4] The resulting pump effect additionally favors the contamination of the interior of the implant. It has been suggested that both the interior contamination of the implant system[3] as well as the micromovements at the IAI[7] are responsible for initial bone resorption. Values for bone loss of up to 1.5 mm in the first year after prosthetic treatment have been observed,[8] especially in cases where the IAI is located at the level of the crestal bone.[9,10] In addition, an internal bacterial colonization of the implant is suspected of being a co-factor for peri-implantitis.[11] Therefore, the ideal 2-piece implant should exhibit a bacteria-impermeable, gap-free connection between implant and abutment, not allowing for any movement or bacterial ingress even during mastication.

Two different methods of evaluating the permeability of 2-component implant systems are described in the literature.[12] However, most of these studies were conducted without the introduction of simulated masticatory forces.[3,13–17] Only a few studies dealt with a leakage test under simulated masticatory forces, which better reflects in vivo conditions.[18–21]

The aim of the present study was to develop a straightforward test setup for bacterial leakage testing of the IAI of 2-piece implant systems under mastication simulation in a universal testing machine. As an uncomplicated test germ,

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TABLE 1

Tested implant system

Variables	Characteristics
Implant specification	PerioType Rapid Implant diameter: 4.1 mm Implant length: 11 mm
Abutment specification	ZircoSeal abutments • straight junction • 1-mm collar height
Implant-abutment-interface design	Straight internal octagonal connection with a cylindrical ring at the implant shoulder
Screw tightening torque according to the abutment manufacturer	30 Ncm

Enterococcus faecium was used under the assumption that its leakage due to insufficient tightness of the IAI can be detected with an indicator agar.

MATERIAL AND METHODS

For the purpose of detecting leaks in the IAI, an experimental setup was designed to permit a visual presentation of apparent leakages.

We tested one type of implant with an internal octagonal butt joint in the IAI with the purpose of rotation protection (Table 1, Figure 1). For the mounting of the implants in the testing machine, they were incorporated into test specimens. For this purpose, a stainless steel tube (length: 50 mm, diameter: 28 mm, wall thickness: 1 mm) was filled with polymethylmethacrylate (PMMA) resin (PalaXpress-transluzent, Heraeus Kulzer, Hanau, Germany) and polymerized. To increase the bond-strength between the resin material and the tube, the inner walls of the tube were pretreated with the Rocatec system (3M ESPE, St Paul, Minn). After that, the resin end face of the specimens was polished. Subsequently, a central hole was drilled perpendicular to the surface, into which the implants were inserted and bonded with PMMA. In accordance with ISO 14801:2007, the implant shoulder was placed 3 mm above the level of the resin surface to simulate peri-implant bone resorption.

A smear of the *E. faecium* was dissolved in 10 ml Tryptic-Soy-Broth (TSB) (Oxoid Limited, Basingstoke, United Kingdom) and incubated for 48 hours at a temperature of 37°C. The resulting suspension was subsequently mixed by a Vortex-mixer (MIX TM 01, Retsch Technology GmbH, Haan, Germany) and poured over the surface of 15 Tryptic Soy Agar plates (Oxoid Ltd, Basingstoke, United Kingdom) in 100 µl doses each. The inoculated agar plates were incubated for 96 hours at a temperature of 37°C. The bacterial layer was washed from the surfaces using 4 ml physiological saline solution (NaCl 0.9%) for each plate. This suspension was centrifuged at 3000 U/min for 10 minutes. Finally, the supernatant was poured off. The obtained bacterial pellets were mixed with 2 ml of glass pearls and 2.5 ml of NaCl and stirred in the Vortex-mixer for 1 minute to produce the final bacterial suspension (BS).

For the preparation of the test suspension (TS), 1 ml of BS was mixed with 9 ml TSB to offer sufficient nutrient supply for the bacteria.

To determine the colony-forming units (CFU/ml), the last three BS- (up to 10^{-9}) and TS- (up to 10^{-8}) dilution series were plated on two kanamycin aesculin azide agar (KAAA) plates (Kanamycin Aesculin Azide Selective Medium, Oxoid) by a spiral

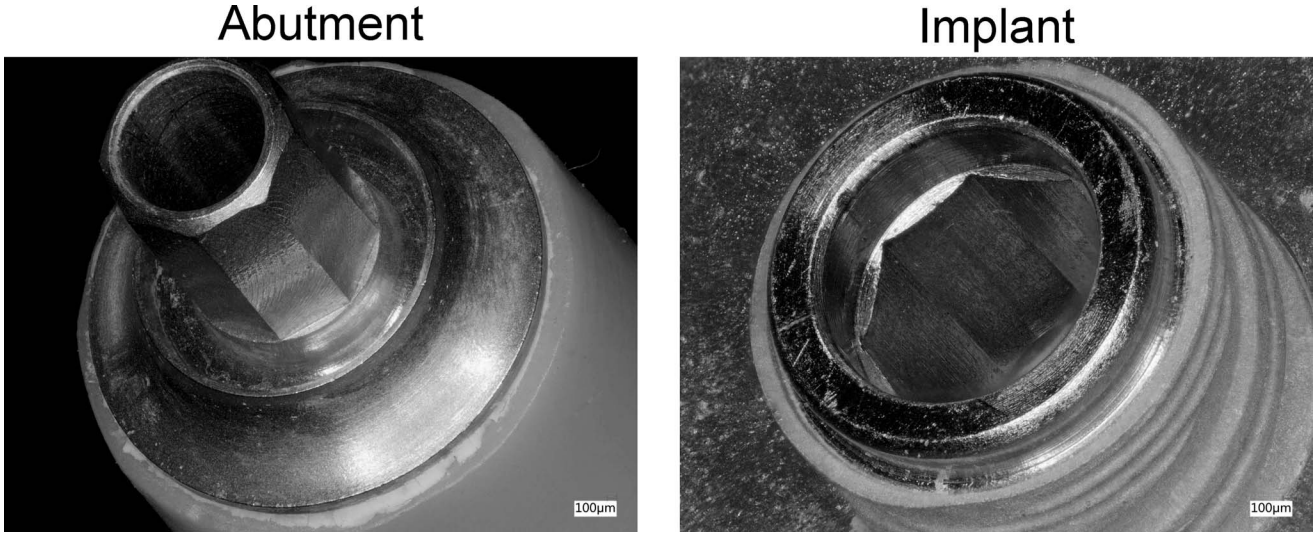

FIGURE 1. Design of the implant-abutment-interface (IAI) of the implants and the corresponding abutments (magnification × ~60).

TABLE 2

CFU-counting of the separate test series (CFU < 20 were excluded from the count)*

	CFU/ml of the dilution series					
	BS			TS		
Test specimen	10^{-9}	10^{-8}	10^{-7}	10^{-8}	10^{-7}	10^{-6}
PerioType 1	–	$5.0/4.3 \times 10^2$	$4.3/3.9 \times 10^3$	–	$5.8/5.4 \times 10^2$	$4.5/4.2 \times 10^3$
PerioType 2	–	$6.5/4.2 \times 10^2$	$4.3/4.1 \times 10^3$	–	$4.2/4.0 \times 10^2$	$3.6/3.5 \times 10^3$
PerioType 3	–	$2.0/2.0 \times 10^2$	$2.0/2.0 \times 10^3$	–	$2.7/2.0 \times 10^2$	$2.3/2.2 \times 10^3$
PerioType 4	–	$2.2/1.9 \times 10^2$	$2.0/1.9 \times 10^3$	–	$2.0/2.0 \times 10^2$	$2.0/1.9 \times 10^3$
PerioType 5	–	$5.1/4.6 \times 10^2$	$3.9/3.6 \times 10^3$	–	$3.7/3.6 \times 10^2$	$3.5/3.3 \times 10^3$
PerioType 6	–	$4.1/3.6 \times 10^2$	$3.1/2.8 \times 10^3$	–	$3.8/3.7 \times 10^2$	$3.1/2.6 \times 10^3$
PerioType 7	–	$3.8/3.4 \times 10^2$	$3.1/2.9 \times 10^3$	–	$3.3/3.3 \times 10^2$	$3.0/2.9 \times 10^3$

*CFU indicates colony-forming units; BS, bacterial suspension; TS, test suspension.

plater (Whitley Automated Spiral Plater 2 (WASP2, Don Whitley Scientific Ltd., Shipley, United Kingdom). Twenty-four hours later, the colonies on the plates were counted, and the mean values of the 2 plates were calculated (Table 2). We injected 2.1 μl of TS into each PerioType-implant.

Subsequently, the abutments were connected to the implants, applying the screw tightening torques proposed by the abutment manufacturer (Table 1). To transfer the masticatory force to the abutments in accordance with ISO 14801:2007, ball-shaped caps were manufactured consisting of zirconium dioxide ceramic (Zenostar, Wieland Dental, Pforzheim, Germany). These caps were engineered in a way that the load axis would cut the implant-abutment-axis 8 mm crestally of the implant shoulder.

To prevent an egress of bacteria via the screw channels and the abutment-cap-interface, we pursued maximum bond strength between the caps and the abutments. For this purpose, the surfaces of the abutments including the screw channels, and the internal surfaces of the zirconia-caps were sandblasted with alumina (50 μm grain size) and conditioned with Monobond Plus (Ivoclar Vivadent, Schaan, Liechtenstein) before cementing. The heads of the screws were covered with cotton pellets and sealed by a flowable composite material (TetricEvoFlow, Ivoclar Vivadent). After that, the caps were cemented to the abutments using a self-adhesive resin material (Maxcem Elite, Kerr Dental, Orange, Calif).

After curing the cement, molds were formed around the test specimens with Parafilm (Pechiney Plastic Packaging, Chicago, Ill). The molds surrounding the implant bodies protruding out of the test specimens were filled with 50°C warm KAAA until their ball-shaped caps were almost completely covered. The KAAA solidified at room temperature after 30 minutes.

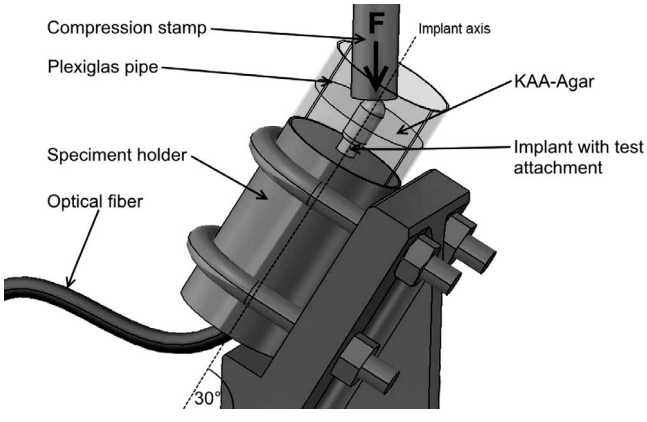

FIGURE 2. Illustration of the experimental test setup.

Immediately afterward, the bacterial permeability of the IAI was examined under static conditions. For this purpose, the samples were incubated for 24 hours at 37°C. Following the incubation, the agar was examined with regard to black staining of its indicator. The staining feature of the indicator in the agar is based on the fact that *E. faecium* is able to hydrolyze Aesculin. The products resulting from this reaction form black iron-phenol-complexes with the iron of the KAAA, which can be seen as black discoloration in the normally transparent agar. To inhibit undesired accompanying bacterial flora, the agar contains kanamycin and sodium azide. Consequently, a high selectivity is achieved.[22]

If no discoloration of the agar was observed in the static phase, the KAAA was removed and the specimen transferred to cyclic load testing.

To keep the agar safely in form around the implant and prevent it from drying out during the dynamic phase, a Plexiglas tube instead of the previously used Parafilm served as mold (Figure 2). The implant body including the zirconium testing-cap was again covered with liquid KAAA at 50°C. After the solidification of the agar, 1 ml of 0.9% saline solution was injected around the implant so that a liquid film between the IAI and the agar could be guaranteed, and the Plexiglas tube was sealed with Parafilm.

All specimens were inserted into a customized steel holding device connected to a universal testing machine (Z005, Zwick Roell, Ulm, Germany) to guarantee an angle of 30° between load axis and implant axis in accordance with ISO 14801:2007 (Figure 2). The specimens were loaded cyclically with a force of 120 N for 1 000 000 cycles.

The incubation temperature of 37°C was sustained by using a water-heating-circulation pump and 2 fan-heat exchanger-units oriented towards the test specimen.

A glass fiber light conductor, aimed towards the test specimen from below, served as light source, ensuring uniform illumination for the optical evaluation of the IAI area. The

Table 3

Negative (–) and positive (+) detections of bacteria discharges at the IAI due to a black staining of the KAAA in the static and dynamic phases*

Test specimen	Static phase (24 h)	Dynamic phase (number of load cycles until bacteria discharge)
PerioType 1	–	+ (41 566)
PerioType 2	–	+ (14 177)
PerioType 3	+	0
PerioType 4	+	0
PerioType 5	–	+ (64 647)
PerioType 6	–	+ (22 329)
PerioType 7	+	0

*KAAA indicates, kanamycin aesculin azide agar.

transparent properties of the plastic in the test specimens made this mode of illumination possible.

A camera (D50, Nikon, Tokyo, Japan) equipped with a macro lens (AT-X PRO D, Kenko Tokina Co Ltd, Tokyo, Japan) was aimed at the test specimens from a lateral position to record the ensuing discoloration. The camera shutter release was controlled by a computer software (DCamCapture, Bernd Peretzke, Ascheffel, Germany) so that 1 picture per hour was taken. The images were subsequently temporally correlated to the load cycles.

The test was stopped as soon as the agar indicator displayed black staining.

Results

Table 3 compiled the numbers of loading cycles after which the bacteria leaked from the IAI. Figure 3 presents an example of the black staining of the KAAA following bacterial leakage through the IAI.

Excluding the 3 specimens that had already demonstrated bacterial leakage in the static test phase, bacterial tightness of the IAIs lasted for 35 680 ± 22 467 cycles on average.

Discussion

Despite the small number of implants evaluated, the results might describe an implant-specific bacterial leakage behavior of the used implant system. This would strengthen the assumption that *E. faecium* injected into an implant in

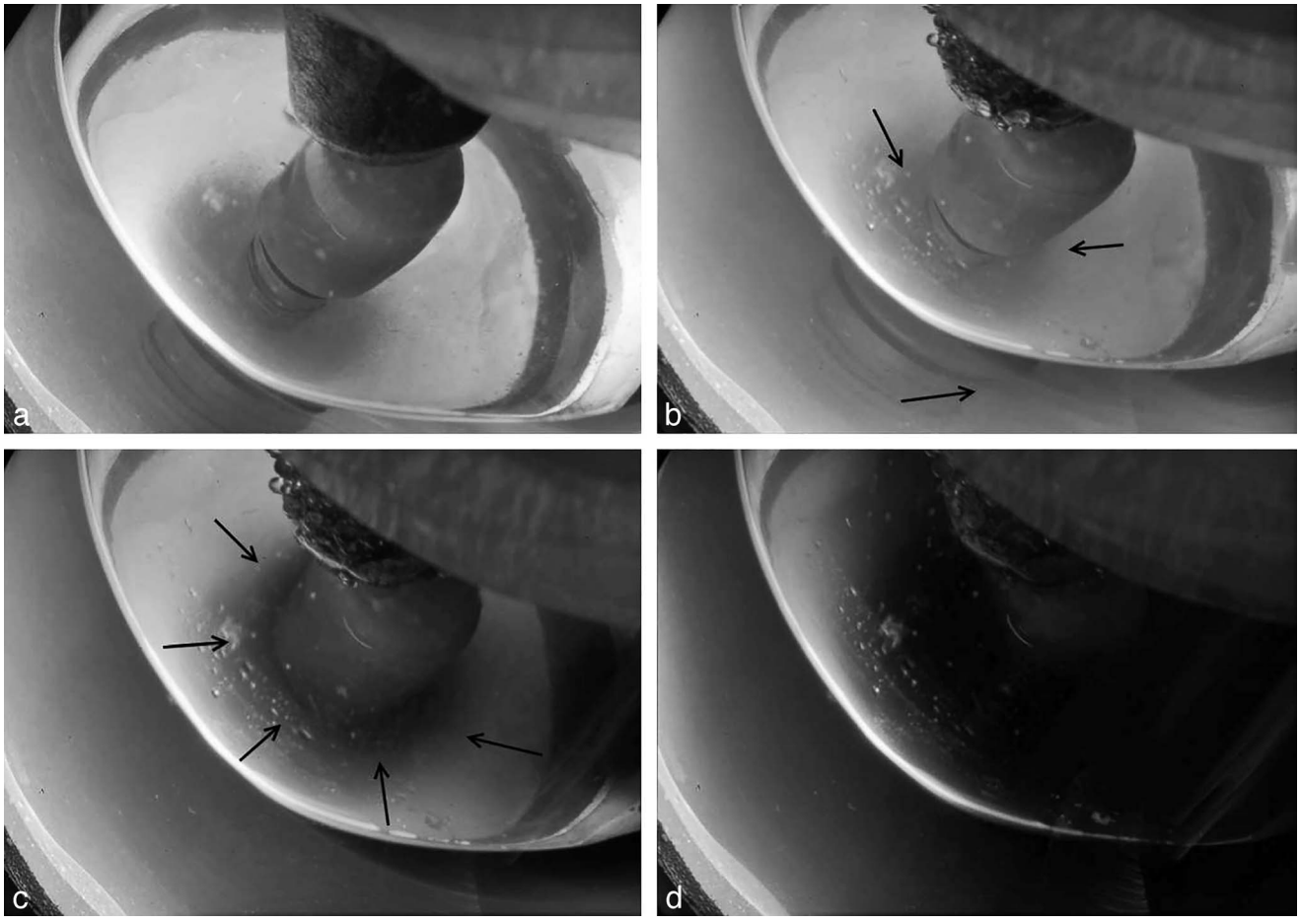

Figure 3. Black staining of the kanamycin aesculin azide agar (KAAA) at the IAI due to its indicator change following bacterial leakage. (a) Fresh translucent agar. (b) First visible indicator changes of the KAAA (arrows). (c) Centrifugal spread of the blackening of the KAAA 5 h after first visible contamination. (d) Complete blackening of the KAAA 10 h after first visible contamination.

combination with KAAA as an indicator of this germ can be used to evaluate the bacterial permeability of IAIs under mastication simulation. Therefore, the primary aim of this study—to design a test method that enables the researcher to visualize and record bacterial discharge from the IAI under simulation of mastication in a reproducible way—was achieved. However, further tests with a higher number of samples are necessary to obtain statistically significant results to confirm this assumption.

The present experimental setup allows the researcher to match the discharge of the bacteria to a relatively exact point in time (± 3 hours) and, thereby, to an according number of loading cycles.

Other studies immersed the test specimens into nutrient or bacterial medium, which only allows an indirect assessment of the bacterial permeability of the IAI. In contrast, our experimental setup does not require the examination of the nutrient medium at a previously specified point in time[18] or after a certain number of load cycles.[19–21]

A possible alternative experimental setup where the bacteria are allowed to migrate from the outside into the implant-abutment-interface is more complicated to handle. Using that method, the specimen has to be immersed into the bacterial medium. To determine the bacterial contamination of the interior of the implant, the test specimen has to be disinfected at its outer surface following the test. Afterward, the abutment has to be removed from the implant. In case of improper disinfection, bacteria might gain access to the interior of the implant, which would lead to false-positive results, or the disinfectant might migrate into the gap at the IAI and thus cause a false-negative result.

A further disadvantage of this method is that the exact point in time of the contamination is not ascertainable.

Continuation of the test after disassembly of the implant-abutment-complex is questionable, since the repeated mounting and dismounting of the abutment screw may have a negative effect on the IAI permeability.[23]

Enterococcus faecium was chosen as test germ for the test setup. This germ is facultative anaerobic and has a size of 0.6–2.0 × 0.6–2.5 μm,[24] comparable to the size of periodontal-pathogenic germs such as *Actinobacillus actinomycetemcomitans*, *Prevotella intermedia*, and *Porphyromonas gingivalis*, which have also been detected in implants.[25] Moreover, substantial evidence has been collected on the presence of the closely related germ *Enterococcus faecalis* inside implants in vivo.[26]

Apart from biomechanical factors such as implant overload,[27] the presence of periodontal-pathogenic germs and their metabolites inside the implants and the associated immune response at the IAI might be responsible for the initial bone resorption after prosthetic treatment.[11–27] Constant bacterial burden at the IAI could also induce severe bone loss and subsequent loss of the implant.[27]

To counteract these risks, manufacturers have chosen different approaches: One of them was the development of the Morse Taper implant-abutment-connection at the IAI. This kind of self-locking connection not only reduces the micromovements of the abutment[4] but also shows an improved sealing of the interior of the implant against bacteria in vitro compared to cone-less implant-abutment-connections.[16,19,20] In vivo, implants with a conical IAI exhibit less resorption of the peri-implant bone after prosthetic treatment.[28]

In some tests, silicone rings achieved a better seal towards the interior of the implant.[3,29] However, in another in vitro test performed under simulated masticatory forces, such implants showed an earlier contamination of the interior of the implant compared to other implant systems without any sealing ring.[18] Steinebrunner et al. assumed that, in this case, both a possible inaccuracy of the polygonal connection (hexagonal in the case of the examined implants), which permits horizontal rotation movements leading to screw loosening as well as a relatively low screw tightening torque of 24Ncm might be responsible.[30]

Accordingly, in the present study, the relatively low number of load cycles until bacterial leakage of the IAI occurred and especially the bacterial leakage of the nonloaded implants could be ascribed to an imprecise fit of the IAI. This presumption has to be further investigated.

Conclusion

Within the limitations of the study, the described experimental setup allows an uncomplicated and conclusive investigation of the bacterial permeability of the implant-abutment interface of 2-component implant systems under the simulation of masticatory forces in a universal testing machine. Additional implant types have to be tested in the future to underscore these results.

Abbreviations

BS: bacterial suspension
CFU: colony forming units
FDP: fixed dental prostheses
IAI: implant-abutment-interface
KAAA: kanamycin aesculin azide agar
PMMA: polymethylmethacrylate
RDP: removable dental prostheses
TS: test suspension
TSB: tryptic-soy-broth

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